SUPPLEMENATAL DATA:

A Corneal Anti-Fibrotic Switch Identified in Genetic and Pharmacological Deficiency of Type III Intermediate Filaments

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Running title: Vimentin is a Fibrosis Target

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. **Vimentin-overexpressing stromal fibroblasts invade injured corneal epithelium**. $Vim^{+/+}$ mice were subjected to corneal alkali injury and treated daily with vehicle (Veh) or 2 mg/kg/d WFA by intraperitoneal injection for 14 days. (A and B) Immunofluorescence staining of vimentin (green) and E-cadherin (red) in uninjured (Unj) corneas or injured corneas of $Vim^{+/+}$ mice treated with Veh or WFA. Nuclei were stained with DAPI (blue). Bar, 150 μm (A). Image of Unj sample is enhanced compared to Veh and WFA samples to reveal low level of vimentin staining in Unj corneal keratocytes (white arrows). (**B**) Selected region (white box) from Veh sample has been magnified to show details of filamentous vimentin expression in invasive fibroblasts found in the epithelium. Bar, 10 μm. Data are representative of two independent experiments (n = 8/group).

Figure S2. Transmission Electron Microscopy of alkali injured corneas. $Vim^{+/+}$ and $Vim^{-/-}$ mice were subjected to corneal alkali injury and treated daily with vehicle (Veh) or 2 mg/kg/d WFA for 14 days. TEM images of injured $Vim^{+/+}$ corneas reveal an abundance of conjunctivalized epithelia evidenced by persistence of globlet cells (a, arrow). Elsewhere in the injured corneas of $Vim^{+/+}$ mice basal cells reveal corneal epithelial characteristics (b, arrow) and presence of hemidesmosomes appear intact (b, inset arrows; epi = epithelium, str = stroma). The epithelia of injured corneas from $Vim^{+/+}$ mice treated with WFA appear normal (c, arrow). Corneas of injured $Vim^{-/-}$ mice also were similarly shown to recover from injury and display corneal epithelial characteristics as well as those from $Vim^{-/-}$ mice treated with WFA (d and e, arrows). Epithelial cells from uninjured corneas of $Vim^{+/+}$ mice (f, arrow) and from $Vim^{-/-}$ mice (g, arrow) are shown for comparison. A keratocyte (g, large arrowhead) in corneal stroma of uninjured $Vim^{-/-}$ mice. Small arrowheads in a-e demarcate the overlying epithelium from basement membrane.

Figure S3. Alignment of desmin and vimentin amino acid fragments used in the molecular modeling studies. The EMBOSS Needle-alignment software from EMBL-EBI was employed to compare the homology between human desmin and vimentin segments from the 2B region contained within the WFA binding site. The cysteine residue (Cys333) that forms a covalent bond with WFA is labeled in red. The amino acids of desmin and vimentin that form hydrogen bonds with WFA are labeled in blue (Gln329) and green (Asp336). The amino acid numbering is for the human desmin polypeptide.

Figure S4. WFA binds soluble recombinant human desmin. Recombinant human tetrameric desmin was incubated for 1 h at 37°C with 5 μM WFA and tryptic digests subjected to analysis by liquid chromatography mass spectrometry. The LC-ESI-MS-MS scans of peptide chromatogram for the tryptic peptides HQIQSYTC'EIDALKGTNDSLMR and HQIQSYTC'EIDALK show an increase by the mass of WFA in the $b7^{+}$ and $y7^{+}$ (singly charged fragment ions) and $b7^{2+}$ and $y7^{2+}$ (doubly charged fragment ions) due to the covalent modification of cysteine residue. The ions observed in the analysis are marked in red and blue.